

Characterization of Polyethersulfone (PES) and Polyvinylidene Difluoride (PVDF)

Resistive Membranes under In Vitro Staphylococcus aureus Challenge

by

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Thesis submitted in partial fulfillment of
the requirements for the degree of Master of Science in the Department of
Biomedical Engineering in the Graduate School
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ABSTRACT

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Abstract

Bacterial colonization of a medical device has been seen to precede clinical infection as well as adversely affect function of the indwelling device. This is a major cause of implant failure with the most common bacterial infections being due to *Staphylococcus aureus*. This strain has been known to be anti-biotic resistant, therefore it is very important to test and find biomaterials with low bacterial adhesion properties to avoid device-associated infections when implanted into the body. In the present study, three specific aims were preformed to characterize the performance of Polyethersulfone (PES) and polyvinylidene difluoride (PVDF) membranes. These polymeric membranes are commonly used in nanofiltration applications in the water and waste water market; therefore they are of high interest for use in the medical device market. PES and PVDF hydrophilic filter disks of 25mm diameter were purchased from Millipore with pore sizes of 0.22 μm . Bacterial migration (N=4), bacterial adhesion (N=4) and outflow resistance (N=4) studies were tested for each filter. Bacteria cultured to a concentration of 1 McFarland (3×10^8 cells/mL) were used for migration and adhesion studies. Migration was tested by pumping bacterial broth through the membranes and collecting the perfusate to quantify the bacterial migration. Adhesion studies were quantified by incubating filters in bacterial broth for 24hrs and plating attached bacteria after detachment by sonication. SEM images were taken for visual analysis of bacteria and

filters. Lastly, outflow resistance was measured by pumping deionized water while recording pressure readings throughout 5 minutes. Results of the studies demonstrated that bacteria did not migrate through both PES and PVDF filters, thus properly filtering *S. aureus* cells. PES membranes were found to have more bacterial adherence to the surface and a lower outflow resistance than PVDF. Both filters could be considered to be used as a part of biomedical devices depending on the specific applications and resistance requirement. However, further studies are needed to inhibit bacterial adherence such as antibacterial coatings or incorporating antimicrobial compounds within polymeric biomaterials. The use of selenium as an antibacterial agent in biomedical devices has sparked a great interest in recent years this, incorporating this in PES and PVDF membranes are the next goal for these studies.

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Acknowledgements

I would like to thank my advisor Dr. Bruce Klitzman and everyone in the Kenan Plastic Surgery Research Laboratories for their encouragement and support. Especial thanks to Lucinda Camras and the Camras Vision Team for their mentorship and guidance. Lastly, I would like to thank my friends and family for inspiring me to keep learning and doing what I enjoy the most.

1. Introduction

1.1 *Bacterial Infections*

Bacterial infections are the most common cause of implant failure therefore being the most common origin of surgery complications. Most cases of infection in critically ill patients are associated with medical devices, increasing their higher risk of infection. According to previous reports, hospitals of all sizes face the problem of bacterial infections and incidents keep rising regardless of hospital size and control measures they take (Panlilio 1992). Several strains of bacteria are very commonly found in the surgical room that could potentially damage the procedure by growing on the surface of the material and stop implant function by infection. Both chemical and physical properties of polymers represent important and complex determinants of adsorption of host proteins and bacterial adhesion and colonization (Delmi 1994). A large percentage of medical devices are of polymeric material due to their excellent mechanical properties such as durability and flexibility. However, once the material is implanted into the body, they get easily colonized by bacteria. Bacteria colonization on medical devices remains one of the most serious complications following implantation, thus investigating the effect of polymers with different bacteria are of high interest in the medical device field. The predominant causative pathogens are bacteria of the Staphylococci genus. *S. aureus* being the main pathogen among infections in the non-medical device associated cases

with internal and external fixation and also one of most prevalent bacterial species in medical device associated infections (Montanaro 2011).

1.1.1 Staphylococcus aureus

Staphylococcus aureus is a gram-positive bacterium commonly found in skin infections and often more severely occurring on surgical wounds, implants, bloodstream or in the lungs. *S. aureus* remains an important cause of nosocomial infections, including nosocomial pneumonia, surgical wound infection and bloodstream infection (Schaberg 1991). In addition, *S.aureus* biofilms have been found on medical devices including urinary catheters, central venous catheters, contact lenses, orthopedic prostheses, heart valves and so forth. It is the most common strain of bacteria found in the hospital environment with 1 million of implant-associated infections per year just in the USA. It has been estimated that it is spent around \$3 billion dollars per year treating biomaterial-associated infections (Kohnen). Bacterial infections of implants are a big factor and one of the leading causes of implant failure, thus this study is highly imperative for prevention of *S.aureus* infections in implants using polymeric membranes. *S. aureus* is also an adept biofilm former, enhancing its virulence capacity. Figure 1 shows an SEM image of single *S. aureus* cells attached to the surface through adhesins or cell wall components, and also an accumulation of multilayered clusters of cells through the production of polysaccharides is seen on the side, suggesting formation of biofilm. *S.*

aureus adherence to a host surface is dependent on ligands such as fibronectin, fibrinogen and collagen. *S. aureus* adheres to these host-tissue ligands via microbial surface proteins known as microbial surface components recognizing adhesive matrix molecules (MSCRAMM). The most important MSCRAMM binding to fibronectin are FnbpA and FnbpB, binding to fibrinogen are clumping factors, and binding to collagen are collagen adhesins; the role of MSCRAMM has yet to be clear in pathogenesis of device associated infections (Darouiche 2001). The Studies have shown increasing prevalence of methicillin resistant that *S. aureus* (MRSA) infections in U.S. hospitals (Panlilio 1992). MRSA outbreaks have been reported as early as the 1960s. These types of bacteria are resistant to many antibiotics, causing life-threatening infections. The bacteria chosen for these studies were an *S. aureus* COL strain. Previous studies have characterized this strain as forming biofilm in vitro and virulent in animal models of endocarditis (Sambanthamoorthy 2008). In addition, the *S. aureus* COL is known to be a less aggressive strain and more sensitive and easily treated.

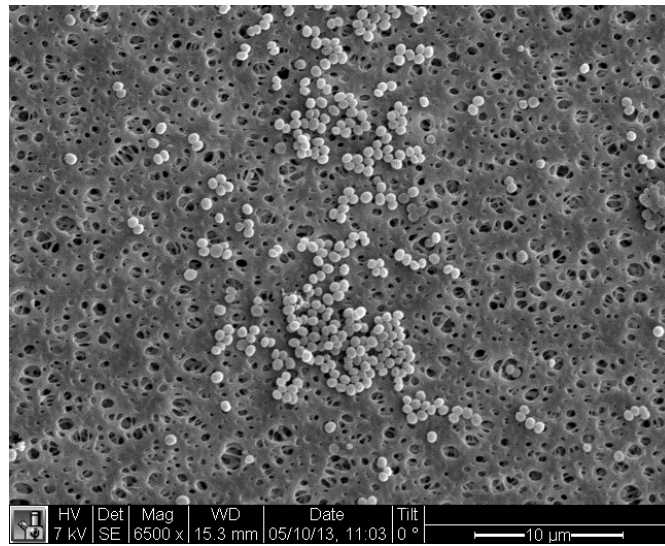


Figure 1: Scanning Electron Microscope Image of *Staphylococcus aureus* at 6500x Magnification

1.2 Membrane Filters in Agricultural and Biological Applications

Membranes are used widely in several applications including as filtration devices for separation of viral particles from biologically proteins, for water and waste water applications, as part as several medical devices such as valves, in dialysis, catheters and ocular drainage devices. Nanofiltration is the process of removing fine particulates from liquid solutions; this is the most important application and the one most useful in the biomedical engineering world. There are several polymers used as commercial membranes for filtration applications. These polymers include: cellulose acetate (CA), polysulfone, polyestersulfone (PES), polyacrylontrile (PAN), and polyvinylidene fluoride (PVDF). Most of the water and wastewater market for

ultrafiltration membranes is now provided by products using PES and PVDF. Therefore, for this study we considered these two filters for their popularity and already successful use in the water filtration application. Table 1 compares PES and PVDF membranes relative to one another, highlighting their different properties. Other sources have stated that hydrophobic materials favor bacterial adherence more than does hydrophilic (Darouiche 2001), therefore for these studies we chose hydrophilic surfaces hoping for less bacterial adherence.

Table 1: PVDF and PES comparison (Pearce 2007)

<i>Parameter</i>	<i>PVDF</i>	<i>PES</i>
Cost	Hi	Med
Ultrafiltration (UF) rating	Med	Hi
Permeability	Med	Hi
Caustic Resistance	Med	Hi
Chlorine Resistance	Hi	Med
Feed Range	Hi	Med
Fiber breaks	Nil-Lo	Med
Membrane Life	Hi	Med-Hi

1.2.1 Polyethersulfone (PES)

PES membranes provide ultrafast filtration of tissue culture media, additives, buffers and other aqueous solutions. The high-throughput, low-protein-binding membrane is used in many ready-to-use sterile filtration devices from Millipore. Figure 2 shows an SEM image of the PES filter provided by the Millipore website.

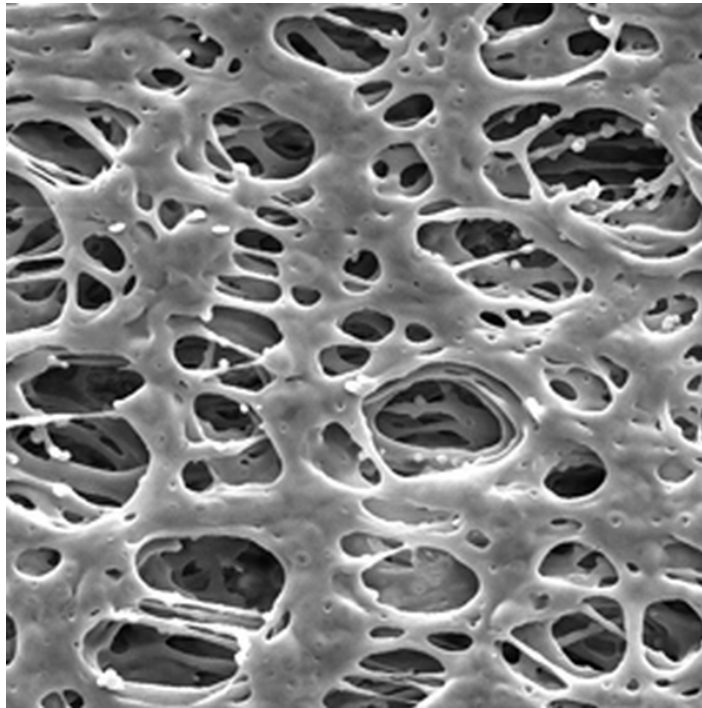


Figure 2: Express PLUS Membrane PES Filter (Millipore GPWP02500).

PLUS membranes by Millipore contain a dull side and a shiny side, which should not affect performance for most applications (Millipore). The shiny side of the membrane is the tighter side, reflecting the asymmetric structure of the filters. For the

bacterial migration studies, sidedness of the filters weren't taken into account for randomization of the filters.

1.2.2 Polyvinylidene difluoride (PVDF)

PVDF membranes provide high flow rates and throughput, low extractables and broad chemical compatibility. Hydrophilic Durapore membranes are said to bind less protein than nylon, nitrocellulose or PTFE membranes (Millipore).

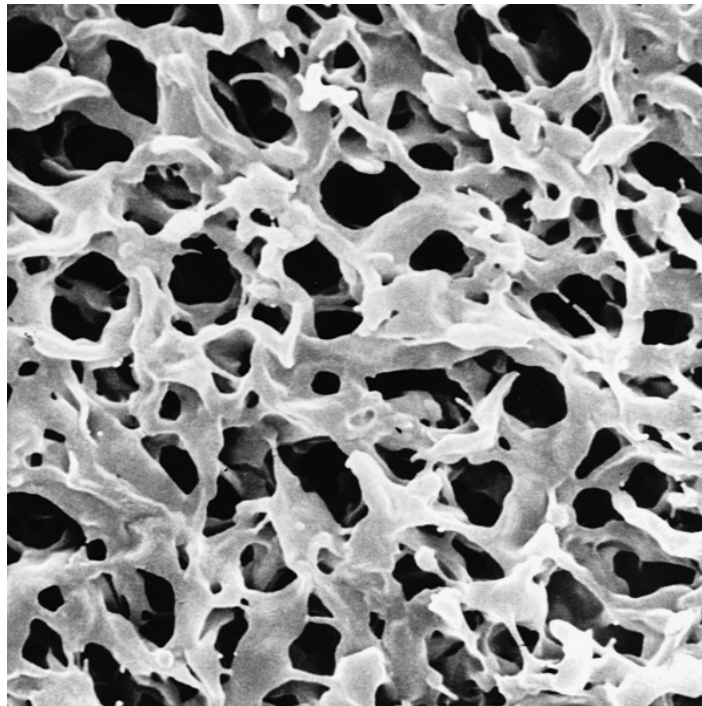


Figure 3: Durapore Membrane PVDF Filter (Millipore GVWP02500).

1.2.1 Polymer Membrane Comparison

There have been a few studies comparing the performance of PES and PVDF membranes, mainly focusing in the filtration efficiency. A study comparing the performance of PVDF and PES in filtering viral suspensions have been reported (Moce-Llivina 2003). This study tested the filtering capacity of both types of membranes by comparing the filtration rate and volume that could be filtered before clogging the system. Results concluded that PES membranes were as affective as the often used PVDF membranes for sewage samples. However, PES allowed higher filtration rate and clogged more slowly. In addition, the group recommended the use of PES membrane filters due to their lower cost and a more efficient method for high recovery of viruses after decontamination by filtration of viral suspensions.

Both membranes in this study were purchased from Millipore (Bedford, MA). Table 2 shows the specifications of the two membranes provided by the company. This table allows for a visual comparison of the different filter parameters used for the experiments.

Table 2: Characteristic Profile of PVDF and PES (Millipore Specifications)

	<i>PVDF</i>	<i>PES</i>
Pore size	0.22 µm	0.22 µm
Thickness	125 µm	≥160µm & ≤185µm
Wettability	Hydrophilic	Hydrophilic
Protein Binding	4 µg/cm ²	42 µg/cm ²
Porosity	70%	Asymmetrical pores
Diameter	25 mm	25 mm
Filter Type	Screen Filter	Screen filter
Bacterial Endotoxins	0.5 EU/mL	0.5 EU/mL

2. Methodology

2.1 Preparation of Bacteria Culture

Staphylococcus aureus COL strain was used for all experiments. The bacteria mixture was prepared using a small sample of frozen stock was obtained and dipped in a tube of Tryptic Soy Broth (TSB) and placed in the incubator at 37°C and 5% CO₂ for 24 hours. Once bacteria proliferated, 10µL of bacterial broth was put into another 9mL TSB tube until bacteria reached exponential growth. After visual examination by turbidity, bacteria were diluted to 1 McFarland standards for an approximated 3x10⁸ bacteria/mL. This same procedure was conducted for all migration and adherence experiments.

2.2 Aim 1: Bacterial Migration

Bacterial migration was measured by allowing bacterial broth perfuse through the two filters by using a syringe pump machine (3-Bracket Bee) connected to filter holders (SterliTech). Perfusate was supplied with two 10mL syringes at 10µL/min to the PES and PEVDF filters in the holders. Filter holders were sterilized by assembling the filter on the filter holder as instructed in SterliTech instruction manual, then by closing the holder but maintain it a quarter turn loose it was placed into a steam permeable paper to prepare for autoclaving. Autoclave was programmed for 121°C (250°F) for 30mins under slow exhaust. After cycle is finished, holders were allowed to cool to room temperature and they were tightened to prevent leakage while perfusing. An N=4 was

used for each filter and one control piece of filter was used by perfusing clean TSB solution.

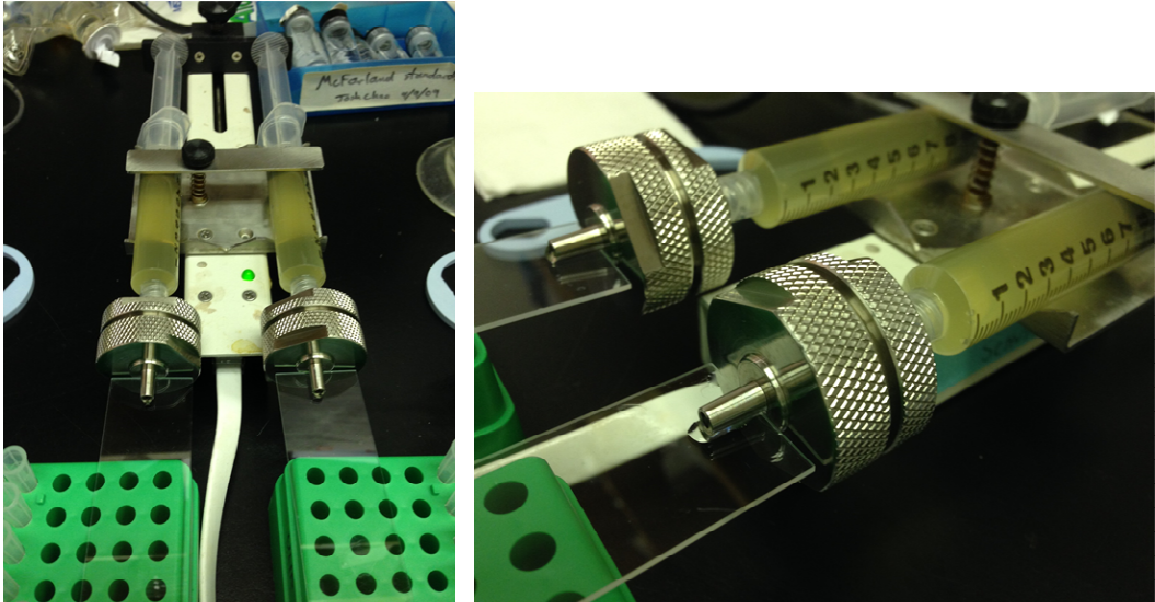


Figure 4: Bacterial Migration Experimental Set Up

2.2.1 Calibration

Experiments to test the performance and reliability of these holders were achieved prior any other experiments done. Sealing of the holders was an initial concern; therefore a system was set up to test if the perfusion would go around the filters instead of passing through the pores of the filters. A thin plastic sheet was cut into a circle with diameter of 25mm to fit the filter holders. Plastic was chosen due to its non-porous surface; hence no water would pass through. Deionized water was used in 10mL syringes when the pump machine was turned on at a flow rate of 100uL/min. After 15

minutes, no water came out from the filter outlet proving that sealing within the filter is leak proof.

2.2.2 Qualitative Analysis

After 15 minutes of perfusion through the filters, one PES and one PVDF filter were fixed and dehydrated in preparation to image with the SEM for a pilot study. More detailed explanation for SEM preparation is explained in section 2.5. Filters were cut in half to capture the differences of the inlet side of the filter and the outlet side.

2.2.3 Quantitative Analysis

After 15 minutes of perfusion through the filter, the bacterial mixture was collected from the sterile glass slide and 100 μ L were diluted one time in 900 μ L of PBS. Dilutions were mixed by pipetting up and down about 20 times and flicking the sterile glass culture tube a couple of times for greater mixture. All these steps were conducted under a cell culture room to keep aseptic techniques and avoid contamination. After one dilution, 10 μ L of PBS dilution was plated into Tryptic soy agar plates and incubated for 24 hours to let the colonies grow. They were counted the next day and recorded for analysis and comparison.

2.3 Aim 2: Bacterial Adhesion

Bacterial adhesion was measured by culturing the filters in 1mL of 1McFarland *S.aureus* broth in a 24 well plate. The 25mm diameter filters were cut in pieces of 8, thus

leaving a surface area of 61.36mm² exposed to the *S. aureus* bacterial broth for bacterial attachment. An N=4 was used for each filter and one control piece of filter was used and cultured in clean TSB solution. The plate was placed in the incubator and left there for 24 hours at 37°C and 5% CO₂. Several other studies have measured bacterial adhesion after an incubation period of as short as 1 hour. This protocol was chosen based on previous studies measuring *S. aureus* in membranes by a group from Texas Tech University (Low 2011). Their studies showed viable cells attached to the membrane surface.

2.3.1 Qualitative Analysis

Qualitative characterization was evaluated by microscopy and image analysis. This was performed by fixing the samples after 24 hours of incubation time. Fixation was done with 4% Formaldehyde and incubated overnight at 4°C. Samples were then prepared for imaging with scanning electron microscopy using a standard biological prep protocol. More detailed explanation of SEM preparation is explained in section 2.5. Membrane pieces were cut in half prior to sputter coating them, hence each side of the filter could be imaged and analysis of all surfaces could be performed.

2.3.2 Quantitative Analysis

Quantitative characterization was evaluated by counting *S. aureus* colonies after membrane incubation. After the filters were incubated for 24 hours, they were washed 3x with 1x PBS. Then, they were placed in sterile glass culture tubes with 1mL PBS and

sonicated them for 10 minutes at 37°C to detach attached bacteria cells. 100µL of this solution was serially diluted twice in 900µL of PBS (1:100). While diluting, mixture was pipetted up and down 20 times and flicked a couple of times to mix vigorously. After the second dilution, 10µL was plated in Tryptic soy agar plates and incubated for 24 hours until colonies were visible and big enough to count. The number of colonies was counted to determine the CFU per disk.

2.4 Aim 3: Outflow Resistance

The outflow resistance studies were done in the Camras laboratory facility. Using a flow pump machine and a 10mL syringe filled with deionized water, membrane resistance was measured at a constant rate of 500µL/min and 250µL/min. Using a former set up provided by the Camras Vision team, filters were placed in a filter holder (SterliTech) joined to the syringe which was attached to the flow pump machine and connected to pressure sensors reading the backed up pressure and resistance given by the filter membranes. Measurements were conducted using only one channel (Channel 1) and one filter at a time. Before starting recording measurements for each filter, initial pressure was confirmed to be around 0. Device calibration and zeroing was done prior to starting with the studies. Readings were recorded 4 times for every second and the average of steadily constant readings for 5 minutes were recorded and considered for the resistance studies.

2.5 Scanning Electron Microscopy

In preparation for the qualitative analysis of the membranes, samples were fixed and dehydrated before being sputter coated with gold prior SEM imaging. As previously stated fixation of the membranes was performed using 4% formaldehyde and incubated overnight. They were later dehydrated by washing them 2 times with 1x PBS for 10 minutes each. After removal, they were washed with 30%, 50%, 70% and 90% and 100% ethanol for 10 minutes each before applying HMDS in the chemical hood for another 10 minutes, this was repeated twice and removed to let the samples completely dry. Once dried, samples were placed in a desiccator before taking them to be sputter coated and prepared for imaging. Several benefits come from sputter coating the samples, such as reducing the microscope beam damage, increasing thermal conduction reducing sample charging, improving secondary electron emission, increasing electrical conductivity (Electron Microscopy Sciences http://www.emsdiasum.com/microscopy/technical/datasheet/sputter_coating.aspx). Images were taken at the Shared Materials Instrumentation Facilities (sMIF) using SEM1 equipment at 2000x and 8000x with a 13-20mm working distance and 7kV accelerating voltage.

2.6 Statistical Analysis

Statistical analysis was performed using a paired t-test. The t-test analysis outputs a t-value and a two-tailed P-value to represent statistical significance of any differences between data sets. Quantity colony counts are reported as mean \pm standard deviation. Significance was determined by statistical analysis (Excel, Microsoft) with values of $P \leq 0.05$ considered significant.

3. Results

3.1 Aim 1: Bacterial Migration

3.1.1 Qualitative Analysis

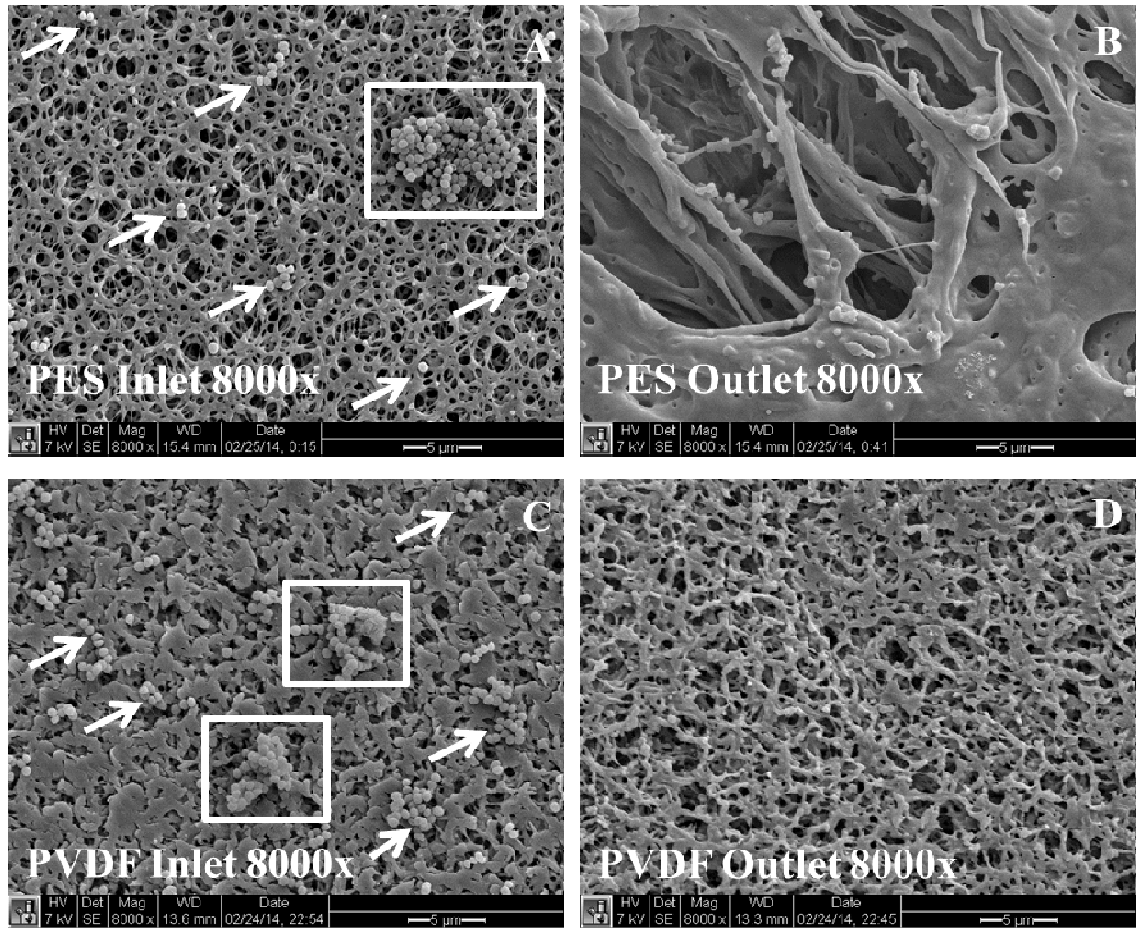


Figure 5: SEM images after *S. aureus* migration studies of inlet and outlet sides of membranes (A, B) PES; (C, D) PVDF. All images taken near the center or middle of filters.

3.1.2 Quantitative Analysis

Table 3: Bacterial Migration Experiments (N=4)

<i>Filter</i>	<i>PVDF</i>	<i>PES</i>
1	0	0
2	0	0
3	0	0
4	0	0

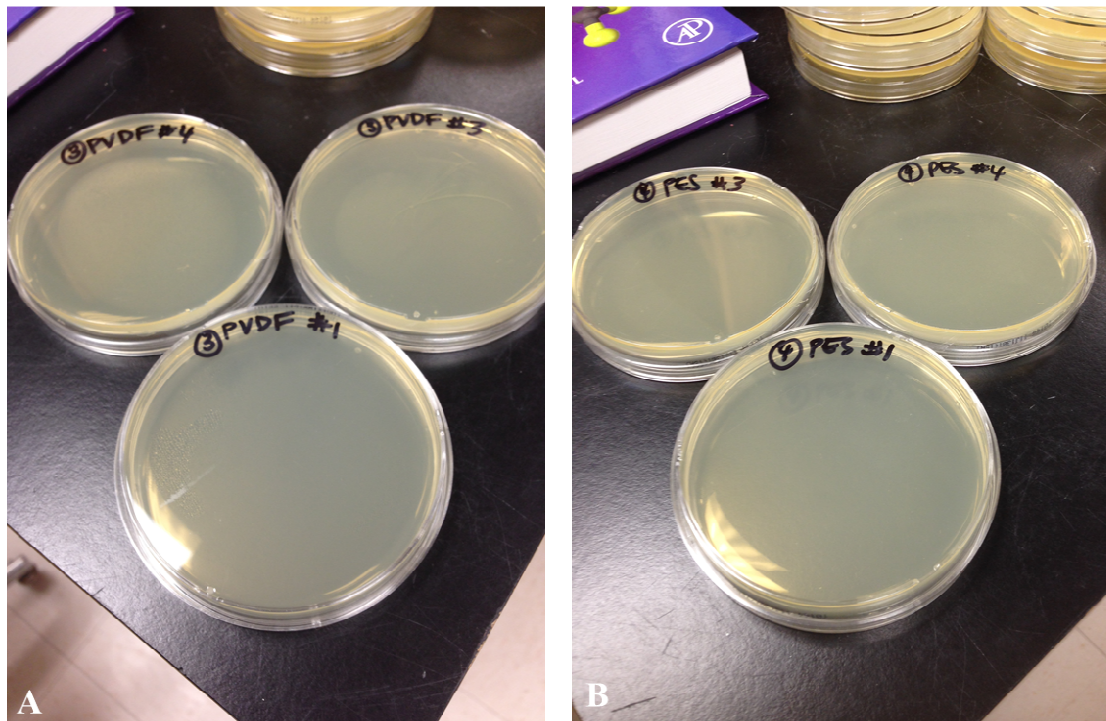


Figure 6: Tryptic Soy Agar plates after 24hrs of incubation at three different dilutions; (A) PVDF Filter #3, (B) PES Filter #4. No colonies seen for both studies.

3.2 Aim 2: Bacterial Adhesion

3.2.1 Qualitative Analysis

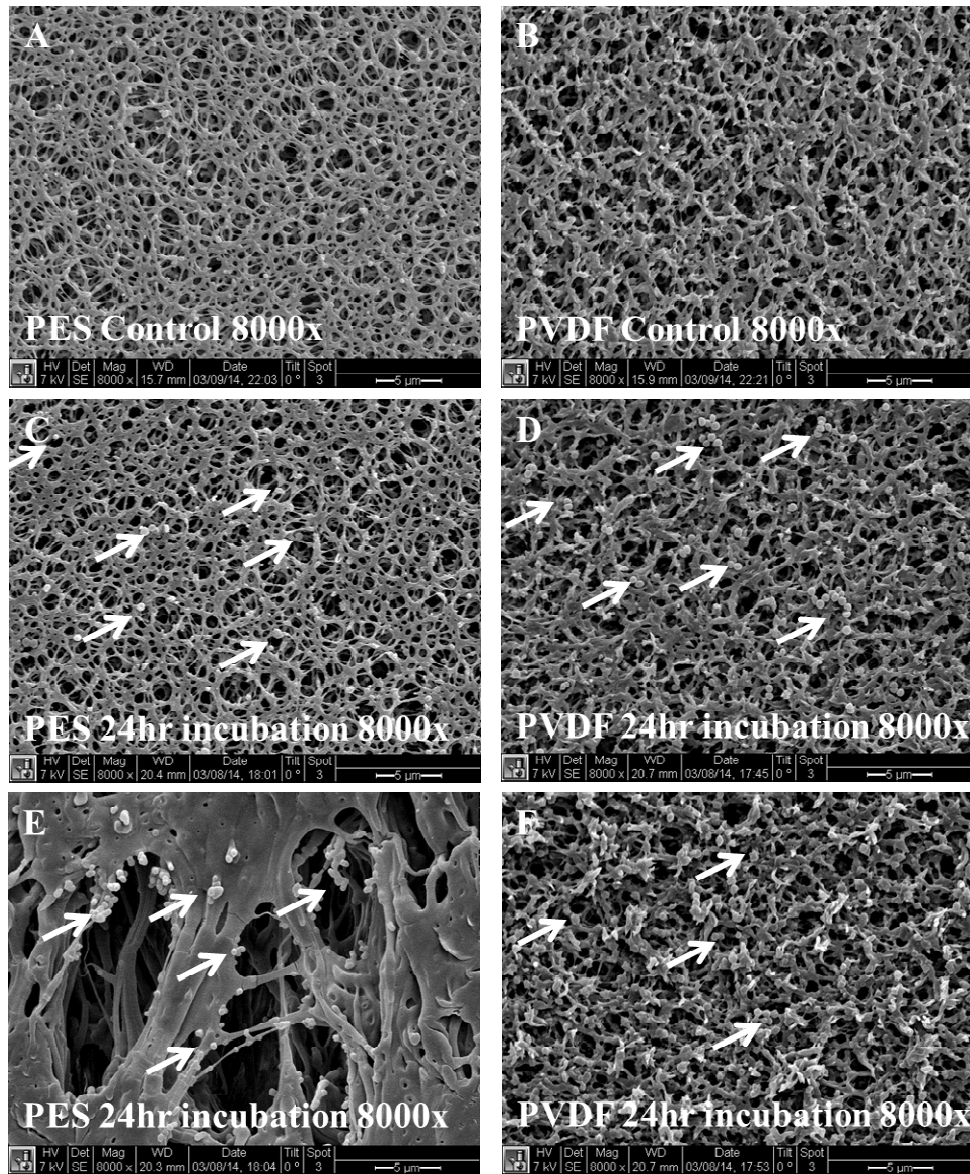


Figure 7: SEM images of a (A, B) control PES and PVDF filter after 24 hours of incubation in clean tryptic soy broth; (C, D) PES and PVDF filter after 24 of incubation in bacterial broth of approximately 1 McFarland; (E, F) Other side of PES and PVDF filter

3.2.2 Quantitative Analysis

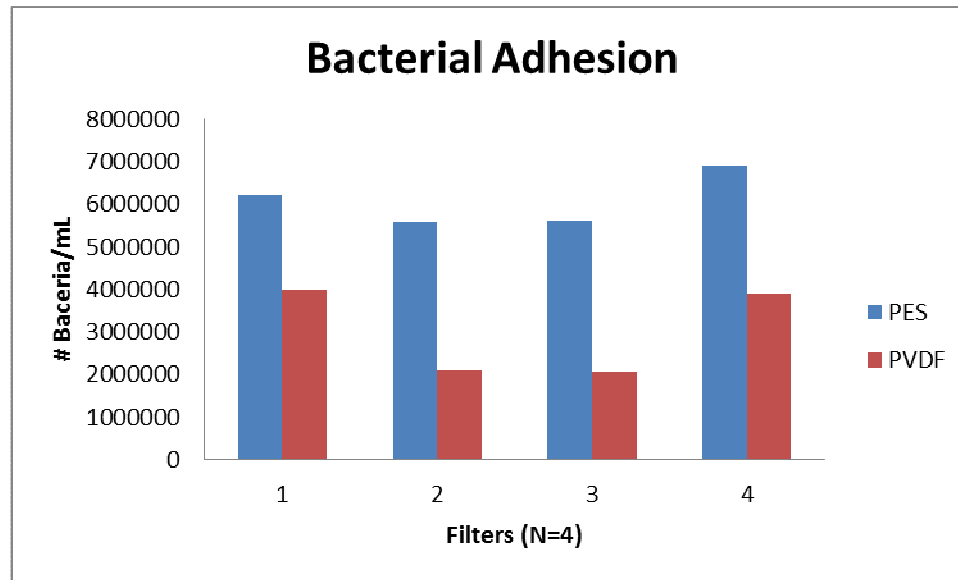


Figure 8: Bacterial adhesion values for PES and PVDF per 1mL (N=4)

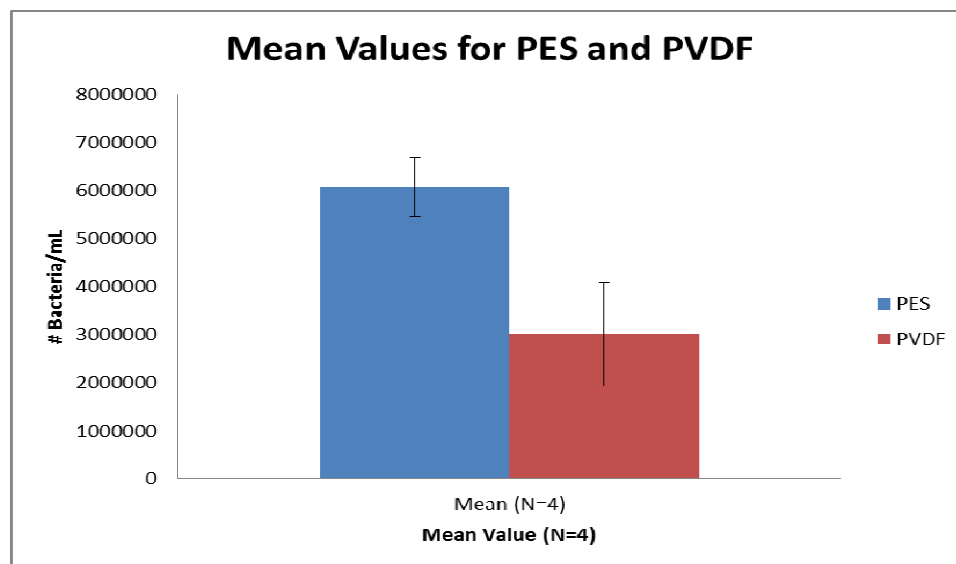


Figure 9: Bacterial Attachment Mean \pm Standard Deviation (PES SD = 622066, PVDF SD = 1080092)

3.3 Aim 3: Outflow Resistance

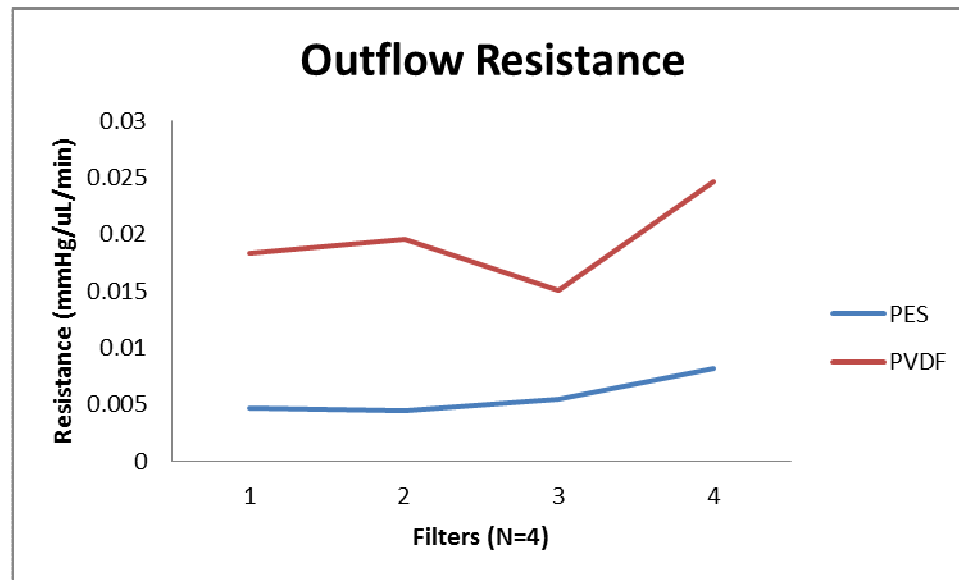


Figure 10: Outflow resistant values for PES and PVDF filters (N=4)

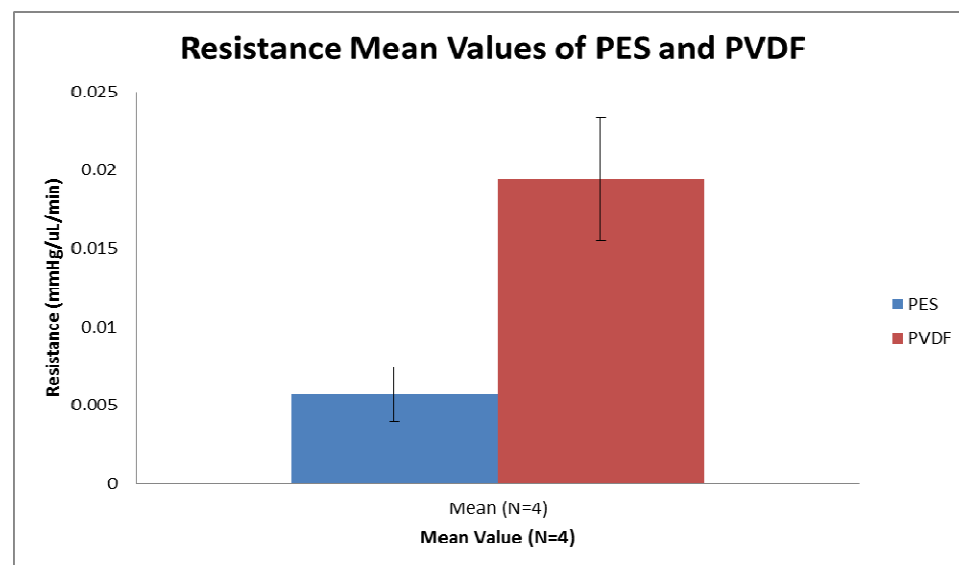


Figure 11: Outflow Resistance Mean \pm Standard Deviation (PES SD = 0.002, PVDF SD = 0.004)

4. Discussion

The present study was conducted to characterize and compare the performance of two different filters membranes by performing *in vitro* bacterial experiments and outflow resistance studies. Polyethersulfone (PES) and polyvinylidene difluoride (PVDF) membranes were chosen for these studies because previous reports have stated that PES and PVDF membranes have good mechanical and chemical properties, specifically in their strength and permeability. PVDF membranes were reported to having great flexibility properties and PES membranes having excellent ultrafiltration ratings, moreover, both of these membranes being the most common polymers used for water filtration applications, are economically reasonable for industry production if needed as a part of an implant.

Bacterial migration experiments showed that both membranes were able to filter out *S. aureus* from a concentration of 1 McFarland after 15 minutes of constant perfusion at 10 μ L/min. Quantifying the perfusate collected allowed to see the number of CFUs that cross the membranes. After testing 4 PES and 4 PVDF membranes and incubating 10 μ L of these dilutions, no *S.aureus* colonies were seen in the tryptic soy agar plates. Visual analysis via SEM was conducted to observe the inlet and outlet side of the membranes. SEM images in Figure 5 showed that the inlet part of both PES and PVDF membranes had bacteria clumps or initial biofilm and single *S. aureus* cells visible on the

filter's surface. Analysis on the outlet side of the filter showed an expected significant difference in *S. aureus* cells. PES membranes have asymmetrical pores, with a shiny (tighter) side and a dull side. Millipore states that orientation of the membranes will not affect filter performance, however, some applications take advantage of the "sidedness" by selecting specific filter orientation. For this study, the membrane orientation was not taken into account to maintain a randomized study. Figure 5 shows the outlet side of the PES membrane as the dull side of the filter, having significant larger pores than the tighter or shiny side. Some bacteria was seen in this side, but *S. aureus* single cells appear to be part of the filter suggesting that they are dead bacteria attached to the filter when the membranes were created at the manufacturing facilities. PVDF membranes did not show *S. aureus* in the outlet side of the membranes, supporting the quantification studies where no bacteria were seen in the perfusate and suggesting that all bacteria was filtered out by the PVDF membranes.

Bacterial adhesion studies were evaluated after a 24 hour incubation period. Figure 7 shows SEM images of both sides of a filter. Filters were cut in pieces of 8 from the manufactured 25mm disks given by Millipore, thus an approximated surface area of 61.36mm² was tested for *S. aureus* attachment. All four PES and PVDF filters showed same characteristics of bacterial attachment in both sides of their surfaces, hence one filter of each were chosen to represent all other images and shown in Figure 6. SEM

images show bacterial cells attached to the surface of the membranes as indicated by the arrows in the images. No *S. aureus* clumps were seen in the filters, suggesting no major attachment or biofilm formation to the surface of the polymer membranes after the incubation period. The Millipore's PES and PVDF membranes are characterized by having low protein binding properties which could explain the low number of *S. aureus* cells seen through SEM imaging. Control samples were also examined under SEM by incubating filters on clean tryptic soy broth for 24 hours. Cultured broth was plated after 24hrs and no colonies were seen to grow after one day in the incubator, supporting that no live bacteria was in the controlled broth used. However, SEM images showed clean membranes with a couple of single *S. aureus* cells in the surface. One speculation is that membranes had some dead *S. aureus* cells already attached when purchased from the manufacturer even after sterilization. Dead bacteria are not harmful or risky to be prone to biofilm production leading to infection. Colony quantification was performed by washing incubated filters three times with PBS to clean membranes from loosely attached cells. Then, after 10 minute sonication in 1mL of PBS, adhered *S. aureus* cells were detached from membrane surfaces. After two dilutions (1:100), 10 μ L of solution was plated and incubated for 24hrs. Tryptic soy agar plates were analyzed by counting colonies and converting values to number of bacteria per 1 mL of solution. Results shown in Figure 8 and 9 present the difference of bacterial cells found in PES and PVDF

membranes. PES showed a higher number of bacterial cells, almost doubled the amount of cells found in PVDF membranes ($P < 0.003$). Millipore's PVDF membrane is reported to have the lowest protein binding properties compared to others ($4 \mu\text{g}/\text{cm}^2$) whereas PES is stated to have ($42 \mu\text{g}/\text{cm}^2$), supporting our bacterial adhesion studies.

Aim 3 was conducted to measure the outflow resistance of both filters and to do a comparison of the filtration performance of the membranes using deionized water. According to Millipore, PES is reported to have a lower resistance and faster filtration capabilities than PVDF. Other papers by Pearce and Moce-Llivina (Moce-Llivina 2003, Pearce 2007) reported that PES allow the filtration of greater volumes of samples than PVDF. Average readings after 5 minutes of demonstrating a stable pressure were taken. Figure 10 and 11 shows the outflow resistance of the filters ($N=4$), indicating that PES has a much lower resistance than PVDF as suspected ($P < 0.0008$). This data confirms previous studies which reported PES as a much faster and efficient filtration device for large volumes of water. The goal of this specific aim was to calculate resistance outflow of Millipore's hydrophilic PES and PVDF membranes to be able to evaluate their ability to filtrate liquid at a measurable pace for applications where rapid open flow is not needed.

4.1.1 Future directions

Further studies on PES and PVDF filters would be beneficial to have a more thorough understanding of their capabilities to be used as part of a medical implant. Avoiding bacterial infections is a major concern in several divisions of medicine, especially in orthopedics, ophthalmology, plastic surgery and medical devices. The next step to this study will be to prevent bacterial adhesion is binding antimicrobial coating to the polymer surfaces. Several nanoparticles have been used as coating on medical device surfaces as antimicrobial agents. In recent years, a lot of interest has sparked in exploring selenium to be used as antibacterial and antimicrobial agent for biological and biomedical systems.

One of the main applications being investigated in recent years have been using selenium as nanoparticles or as organoselenium monomers covalently attached to compounds in membranes to be applied as antimicrobial or antibacterial coating for medical devices. Previous research suggested that selenium-enriched probiotics have shown to strongly inhibit the growth *E.coli in vivo* and *in vitro*. A group in Texas examined the effect of Organoselenium in RO membranes to characterize their antibacterial activities *in vitro* for waste water filtration applications (Vercellino 2013). These studies reported novel results where bacterial biofilm was inhibited, thus being an excellent candidate for an antimicrobial agent in medical devices. Selenium catalyzes

superoxide production by redox reaction, which compromises bacterial cellular membranes to interact with fatty acids hence, unable to attack the membranes (Fridovich 1983). Furthermore, studies testing the bacterial inhibition of selenium nanoparticles *in vitro* have also been recently reported. Tran and Webster tested selenium nanoparticles' inhibition to *Staphylococcus aureus* bacteria. After synthesizing selenium nanoparticles using different methods, results showed that selenium nanoparticles created by colloidal synthesis method with an average of 100nm diameter, inhibited *S. aureus* by up to 60 times compared with no treatment surfaces. The inhibitory effects of the nanoparticle coating at 7.8, 15.5 and 21ug/mL were seen to kill about 40% of the bacteria at 3, 4 and 5 hours (Tran and Webster 2011). Later studies by the same group experimented by testing the bacterial inhibition of selenium nanoparticles in different polymers including polyvinyl chloride, polyurethane and silicone; which are the most common polymeric materials used for continuously infected medical devices. It was seen that the reduction of bacteria growth directly correlated with the density of selenium nanoparticles on the coated substrate surfaces, suggesting that selenium nanoparticles are a great alternative coating for implants. Results from both studies demonstrated that selenium nanoparticles are a novel anti-bacterial polymeric coating material that functions without the use of any antibiotics and should be further studied for potential use in membrane filters and biomedical devices. Overall, selenium is gaining interested in

antibacterial technology which might be able to reduce costs and post-surgical infections and complications (Vercellino 2013).

In addition, further studies in using animal models should be also conducted to examine the foreign body reaction of these filters when implanted in the body. As previously said, PES and PVDF membranes possess an excellent filtration rate for incorporation in medical devices. Depending where the device and membrane are located, it can form and attract more bacteria, thus being highly prone to infection. For example, catheters are very common in forming biofilm around their surfaces due to the location of the device in the body. However, other parts in the body such as the eye or the mouth might have natural antimicrobial components such as in the tears and saliva that can inhibit bacterial formation in medical surfaces. Therefore, *in vivo* studies testing both membranes at different locations including in the ocular region, in the mouth, subcutaneously or intramuscular to test for bacterial infection.

5. Conclusion

This study focused on characterizing the performance of PES and PVDF membranes under *Staphylococcus aureus* challenge. In addition, it measured the outflow resistance to test filtration performance of the two filters. PES and PVDF membranes were chosen for these studies due to their excellent mechanical and chemical properties. Providing the majority of commercial membrane filters for the water and waste water market, these two polymers are excellent for the use in medical devices. A concern when implanting polymers in the body are bacterial infections. Therefore, testing the bacterial migration and adhesion is of great importance to characterize these filters. Results showed that *S. aureus* adheres to PES surfaces in greater numbers than to PVDF membrane surfaces, moreover migration studies showed that both membranes filtered out bacterial cells equally. Lastly, the outflow resistance was measured to compare membrane filtration performance. PES showed a much lower resistance than PVDF suggesting faster and more efficient filtration characteristics. In conclusion, after relative comparison of both PES and PVDF filters, each membrane showed benefits for different specific applications. Applications where filtration speed and performance is imperative PES would be the best choice; however, bacterial attachment would be of concern for possible biofilm formation. Therefore, future studies testing coatings as an antibacterial

agent is of high interest, with major focus on selenium nanoparticles and organoselenium compounds.

Table 4: Performance Profile of PVDF and PES

<i>Specific Aims</i>	<i>PVDF</i>	<i>PES</i>
1. Bacterial Migration	None	None
2. Bacterial Adhesion	Low	High
3. Outflow resistance	High	Low

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